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Research Paper

Using microRNAs as Novel Predictors of Urologic Cancer Survival: An Integrated Analysis

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ABSTRACT

Background: MicroRNAs(miRNAs) are involved in the formation, maintenance, and metastasis of urologic cancer. Here, we aim to gather and evaluate all of the evidence regarding the potential role of miRNAs as novel predictors of urologic cancer survival.
Methods: A systematic review was performed to identify and score all of the published studies that evaluated the prognostic effects of miRNAs in kidney (KCa), bladder (BCa) or prostate cancer (PCa). Where appropriate, the summary effects of miRNAs on urologic cancer were meta-analysed. The reliability of those results was then further validated by an integrated analysis of the TCGA cohort and miRNA panel.
Results: Of 151 datasets, 80 miRNAs were enrolled in this systematic review. A meta-analysis of the prognostic qualities of each miRNA identified an objective association between miRNA and prognosis. miR-21 was identified as an unfavourable miRNA with the overall survival (HR:2.699, 1.76–4.14, $P < 0.001$) across various prognostic events. Our further meta-analyses, integrating a parallel TCGA analysis, confirmed these partial previous results and further revealed different summary effects, such as the moderate effect of miR-21 in BCa. The refined miRNA panel (KCa-6: miR-27b, −942, −497, −144, −141 and -27a) was more capable of predicting the overall survival than was any single miRNAs included in it (HR: 3.214, 1.971–5.240, $P < 0.01$).
Conclusions: A miRNA panel may be able to determine the prognosis of urologic tumour more effectively and compensate for the unreliability of individual miRNA in estimating prognosis. More large-scale studies are therefore required to evaluate the unbiased prognostic value of miRNAs in urologic cancer effectively.
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1. Introduction

The evaluation of cancer prognosis is necessary for treatment selection, patient counselling, the design and analysis of clinical trials, and understanding the disease process and outcome [1]. Cancer prognosis is interrelated with diverse factors including the physical status of the patient, pathological stage or clinical stage of tumour, disease development, and clinical interventions [2–4]. It is yet far from satisfied that accessing the prognosis by the current prediction tools. For prostate cancer (PCa), there is no consensus about whether prostate specific

Abbreviations: RNA, ribonucleic acid; miRNA, microRNA; KCa, kidney cancer; BCa, bladder cancer; PCa, prostate cancer; TCGA, The Cancer Genome Atlas; OS, overall survival; PSA, prostate specific antigen; WHO, World Health Organization; NMIBC, non-muscle-invasive bladder cancer; PRISMA, Preferred Reporting Items for Systematic Review and Meta-analysis; DFS, disease free survival; PFS, progression free survival; RFS, relapse free survival; CSS, cancer/disease specific survival; BCR-FS, biochemical recurrence free survival; HR, Hazard Ratio; 95%CI, 95% Confidence interval; T, size or direct extent of the primary tumour; LNM, lymph nodes metastasis; DM, distant metastasis; G, histology grade; Gleason, gleason score; Stage, TNM stage; GEO, Gene Expression Omnibus; NOS, Newcastle-Ottawa quality assessment scale; FFPE, formalin fixed paraffin-embedded; qRT-PCR, quantitative real-time polymerase chain reaction; qPCR, quantitative polymerase chain reaction; ISH, in-situ hybridization; AT, advanced T-stage; HG, higher histologic grade; AS, advanced TNM stage; OR, odds ratios; SE, standard error; TCGA-BLCA, bladder cancer in TCGA chort; TCGA-KIRC, kidney clear cell carcinoma in TCGA chort; TCGA-KIRP, kidney papillary cell carcinoma in TCGA chort; TCGA-KICH, kidney chromophobe carcinoma in TCGA chort; TCGA-PRAD, prostate adenocarcinoma in TCGA chort; IQR, interquartile range; CHN, Chinese; non-CHN, Non-Chinese; AUC, the area under the curve; ROC curve, receiver operating characteristic curve; BRISQ, Biospecimen Reporting for Improved Study Quality; REMARK, Reporting Recommendations for Tumour Marker Prognostic Studies; TRIPOD, Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis.

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Research in Context

Evidence Before this Study

In this study, the PubMed, Cochrane Library and Web of Science electronic databases were systematically searched for studies by using "microRNA with prostate carcinoma or bladder carcinoma or kidney carcinoma" as keywords to combine screening. The literature search was last updated on November 21, 2017.
After removing duplicate records, we screened titles and abstracts to identify relevant articles. Relevant studies must meet the following criteria before being included: [1] the published miRNA studies focused on kidney carcinoma or bladder carcinoma or prostate carcinoma; [2] the studies must have explored the association between the expression level of any single or combination of miRNAs and any of the following types of survival analysis: overall survival; disease-free survival; progression-free survival; relapse-free survival; cancer − /disease-specific survival and biochemical recurrence-free survival. The studies had to provide an explicit HR (Hazard Ratio), 95%CI (Confidence interval) and *P* value or a survival curve from which we could extract the HR, CI and P value; [3] eligible studies without any survival analyses had to contain the following clinicopathologic characteristics: T stage (the size or direct extent of the primary tumour), lymph node metastasis, distant metastasis, histology grade, prostate specific antigen, Gleason score and TNM stage. Clinicopathologic characteristics had to be grouped by miRNA expression level; [4] the full text was available. Correspondingly, the study was excluded based on the following criteria: [1] duplicate publications; [2] an animal or non-clinical study; [3] reviews, case reports, letters, editorials, or expert opinions; [4] studies not grouped according to miRNA expression level; [5] studies on the genetic alteration of miRNAs, including polymorphisms and methylation patterns; and [6] clinical and survival analysis data obtained from The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO) or other tumour databases.
Newcastle-Ottawa quality assessment scale (NOS) was using to access the quality of the included studies. In order to further quantify its prognostic ability, we scored the miRNA by its prognostic event, including clinical and survival events. Publication bias in this meta-analysis was evaluated with either Egger's test or Begg's test.Added Value of this Study

Of 151 datasets, 80 miRNAs were enrolled in this systematic review. A meta-analysis of the prognostic qualities of each miRNA identified an objective association between miRNA and prognosis. miR-21, which was the most frequently studied miRNA and had high consistency among these studies, was identified as an unfavourable miRNA with the overall survival (HR:2.699, 1.76–4.14, $P < .001$) across various prognostic events. Our further meta-analyses, integrating a parallel TCGA analysis, confirmed these partial previous results and further revealed different summary effects, such as the moderate effect of miR-21 in bladder carcinoma. The refined miRNA panel (KCa-6: miR-27b, −942, −497, −144, −141 and -27a) was more capable of predicting the overall survival than was any single miRNAs included in it (AUC:0.755, HR: 3.214, 1.971–5.240, $P < .01$) and nearly the same as that of pathologic stage (AUC:0.763, HR: 4.502, 2.719–7.454, $P < .01$). Patients who were separated by integrating KCa-6 and staging had significantly different prognoses ($P < .0001$).Implications of all the Available Evidence

In this study, we have gathered almost all of the prognostic data regarding the association between miRNA and urologic cancers. A miRNA panel may be able to determine the prognosis of urologic tumour more effectively and compensate for the unreliability of individual miRNA in estimating prognosis. Researchers can draw attention to large-scale studies with a standardized methodology that assess both single and multiple miRNAs and, it is hoped, evaluate the unbiased prognostic value of miRNAs in urologic cancer effectively.

antigen (PSA) tracking can effectively evaluate the risk of death [5, 6]. For bladder cancer (BCa), the prognostic performance and reproducibility of the 1973 and 2004/2016 WHO grading classification systems in non-muscle-invasive BCa (NMIBC) is still debated [7, 8]. Therefore, it is necessary to improve the accuracy and timeliness of disease management by refining the current prognostic judging systems and strategies.

An unparalleled achievement in cancer genomics has been achieved due to the rapid evolution and development of gene sequencing. All kinds of cancer-associated molecular biomarkers, ranging from coding genes [9–11] to non-coding genes [12, 13], have been identified in various biologic and clinical aspects. microRNA(miRNA) is one kind of non-coding RNAs (19–25 nucleotides) which can silence RNA and post-transcriptionally regulate gene expression, playing an essential role in different cancers [14, 15]. Some miRNAs abnormally and dysfunctionally expressed in cancer, and they serve as tumour suppressors that target oncogenes or oncomiRNAs that target suppressor genes [16]. Benefitting from recent technical advances in the methods used to examine miRNA expression and function, miRNAs have been widely studied and applied in cancer diagnosis, classification, and prognostic indication [17–19]. Further, several miRNA-targeted therapeutics have already entered clinical trial phase and are being tested at different centres [20–22]. It is reasonable to believe that miRNAs will be fully transformed from bench to bedside in the near future.

Remarkably, it is now clear that miRNAs are vital regulators in urologic cancers [13, 23–26]. Approximately 18 meta-analyses concerning the roles of miRNAs in urologic cancers have been published over the past five years. All of these studies focused on a survival analysis of a single cancer without a reasonable subgroup, while Only 40% of them considered the prognosis of urologic cancer [27–30]. Here we carried out a comprehensive integrated analysis to systematically identify and investigate the potential roles of all miRNAs that were ever included in prognostic studies on human urologic cancer to better understand the relationship between miRNAs and urologic cancer prognosis.

2. Materials and Methods

2.1. Search Strategy

This report has been structured based on the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines [31]. The PubMed, Cochrane Library and Web of Science electronic databases were systematically searched for studies in English that analysed the associations between miRNA and the prognoses of three main human urologic cancers: KCa, BCa and PCa. The literature search was last updated on November 21, 2017.

The following search algorithms were used:"((microRNA OR micro RNA OR micro ribonucleic acid OR miRNA) AND ((prostate carcinoma OR prostate carcinomas OR prostate cancer OR prostate cancers OR prostate tumour OR prostate tumours) OR (bladder carcinoma OR bladder carcinomas OR bladder cancer OR bladder cancers OR bladder tumour OR bladder tumours)) OR (kidney carcinoma OR renal carcinoma OR kidney carcinomas OR renal carcinomas OR kidney cancer OR renal cancer OR kidney cancers OR renal cancers OR kidney tumour OR renal tumour OR kidney tumours OR renal tumours)) AND (Humans [Mesh] AND English[lang]))".

2.2. Eligibility Criteria

After removing duplicate records, we screened titles and abstracts to identify relevant articles. Relevant studies must meet the following criteria before being included: [1] the published miRNA studies focused on KCa or BCa or PCa; [2] the studies must have explored the association between the expression level of any single or combination of miRNAs and any of the following types of survival analysis: overall survival (OS); disease-free survival (DFS); progression-free survival (PFS); relapse-free survival (RFS); cancer−/disease-specific survival (CSS) and biochemical recurrence-free survival (BCR-FS). The studies had to provide an explicit HR (Hazard Ratio), 95%CI (Confidence interval) and *P* value or a survival curve from which we could extract the HR, CI and P value; [3] eligible studies without any survival analyses had to contain the following clinicopathologic characteristics: T stage (the size or direct extent of the primary tumour), LNM (lymph node metastasis), DM (distant metastasis), G (histology grade), PSA (prostate specific antigen), Gleason (Gleason score) and Stage (TNM stage). Clinicopathologic characteristics had to be grouped by miRNA expression level; [4] the full text was available.

Correspondingly, the study was excluded based on the following criteria: [1] duplicate publications; [2] an animal or non-clinical study; [3] reviews, case reports, letters, editorials, or expert opinions; [4] studies not grouped according to miRNA expression level; [5] studies on the genetic alteration of miRNAs, including polymorphisms and methylation patterns; and [6] clinical and survival analysis data obtained from The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO) or other tumour databases. Three authors (Zhan, Zheng and Huang) identified eligible studies, and any contested articles were adjudicated by the two other authors (Chen and Guo). Disagreements were resolved with discussion.

2.3. Quality Assessment

Quality assessment, a necessary step in systematic reviews, should be incorporated into the synthesis of cancer prognosis evidence [32]. Here, the quality of the included studies was scored independently by two authors (Zheng and Huang) and confirmed by two other authors (Guo and Zhong) using the Newcastle-Ottawa quality assessment scale (NOS) [33]. A study can be awarded a maximum of 9 points (which contained three parts: selection, comparability and outcome) for all numbered items within the NOS; a score of 6 points or above indicated high quality.

2.4. Data Extraction

A standardized table was developed by two authors (Guo and Zhong) according to the CHARMS checklist [34]. Another author (Chen) supervised the process independently and provided consensus in times of disagreement. The following items were extracted for all included articles: [1] publication details, including the title, first author, year of publication, country and continent; [2] characteristics and details of the variables studied, such as the names of the miRNA, the cancer site (e.g., bladder), the detected sample size, the kind of sample, the miRNA quantitative analysis methods, and the size of the high and low expression group; [3] clinicopathologic characteristics: an advanced T stage (AT,T2/T3-T4), LNM, DM, a higher histologic grade (HG,G2-G3 in BCa and G3-G4 in KCa), an advanced TNM stage (AS, Stage III-IV), prostate specific antigen (PSA ≥ 10 and Gleason score ≥ 7) in both comparison groups and its original *P* value; and [4] survival analysis details, including the type of analysis (univariate, multivariate), the follow-up time, the HR, and the corresponding 95% CI and P value.

All cohorts were used only once, and we extracted data from a broader classification of cancers (e.g., bladder cancer) rather than their sub-classifications (e.g., muscle invasion bladder cancer). Due to the inconsistent annotation system of the included miRNAs (e.g., miR-200c and miR-200c-3p), miRbase was used to normalize the name of the miRNAs [35]. Two studies reported a contradictory sample size, and one permitted the extraction of only the data that were found in the full text [36]; one final paper was completely excluded [37]. When the high expression group was defined as the control group in the survival analysis, the HR was replaced by its reciprocal. Whenever multiple miRNAs were combined into a single dataset to study the miRNA panel, we only extracted the HR respective to each constitutive miRNA instead of extracting the summary HR. On those occasions when we had to calculate the HRs ourselves, Engauge Digitizer 4.1 was used to calculate the HR and the corresponding 95% CI based on the available Kaplan–Meier curves [38, 39].

2.5. Score by the Prognostic Event

To quantify its prognostic ability, we scored the miRNA by its prognostic event, including clinical (AT, LNM, DM, HG, AS, PSA and Gleason scores) and survival events (OS, DFS, PFS, RFS, CSS and BCR-FS). Each clinical event gained 1 point, and each survival event gained 2 points if the *P* value was <0.05 and the odds ratio (OR) or the hazard ratio (HR) was <1, whereas each even lost 1 point and 2 points, respectively, if the P value <0.05 and the OR or HR >1. The event was assigned a 0 if the P value ≥0.05. The clinical and survival event scores were added together for each dataset to define the prognostic effect of miRNA as favourable, moderate or unfavourable (favourable refers to a score >0; moderate refers to a score of 0; unfavourable refers to a score <0) and thereby obtain a general understanding of the miRNA signature.

2.6. Statistical Analysis

All meta-analyses were performed with Stata12.0 (StataCorp LP, College Station, TX, USA). A random effect model (the DerSimonian-Laird method) was applied in this analysis. According to the bivariate clinical event variables, ORs with 95% CIs were calculated and summarized. The log HR and standard error (SE) were used to describe the survival results [39]. To assess the heterogeneity of the included studies, Q, I^2 and tau-squared statistics were used ($P < 0.05$ and/or $I^2 > 50\%$ were considered statistically heterogeneous) [40]. The potential publication bias was assessed using Begg's test whenever there were >10 included studies. Otherwise, Egger's test was performed. *P* values < 0.05 were considered statistically significant.

Moreover, other statistical tests were performed using SPSS, version 22.0 software (SPSS, Chicago, IL, USA). Significant differences between the groups in Table S5 were assessed with the Chi-squared test or non-parametric tests (Mann-Whitney U). Significant differences between the groups in Figs. S5 and S8 were assessed with Student's *t*-test or non-parametric tests (Mann-Whitney U). The Kaplan-Meier method and Cox's proportional hazards regression model were used to calculate OS or RFS, and differences were analysed with a log-rank test. $P < 0.05$ was considered statistically significant.

2.7. Bioinformatic Data Mining of miRNA with TCGA

The expression level and prognostic potential of 4 shared miRNAs (miR-21, miR-34a, miR-141 and miR-203) were validated with the TCGA dataset. Relevant miRNA expression and clinical data on urologic cancers, including bladder cancer in the TCGA cohort (BLCA), kidney clear cell carcinoma in the TCGA cohort (KIRC), kidney papillary cell carcinoma in the TCGA cohort (KIRP), kidney chromophobe carcinoma in the TCGA cohort (TCGA-KICH) and prostate adenocarcinoma in the

TCGA cohort (PRAD), were downloaded from UCSC Xena (http://xena.ucsc.edu/, up to December, 22,2017).

2.8. The Cox Model of Multi-miRNAs

To model the relationship between the survival time of a certain patient and miRNA expression, the Cox Proportional Hazard regression model was constructed with SurvMicro within the TCGA kidney clear cell carcinoma cohort (SurvMicro TCGA-KIRC, n = 217) (http://bioinformatica.mty.itesm.mx:8080/Biomatec/Survmicro.jsp) [41]. The prognostic index is the linear component of the Cox model, and it was used to generate the risk group. The samples were split at the median after being ranked by the prognostic index (a higher score indicated a higher risk and poorer prognosis). Kaplan-Meier and log-rank tests were performed to interpret differences in the survival distributions between different groups. All of the miRNAs were initially included in the Cox multivariate fitting model, i.e., KCa-35. The backwards method was then applied to exclude unrelated miRNA variables step by step according to the *P* value of each β_i coefficient, until all of the residual $P_{\beta i} < 0.05$ and miRNA that were expressed differentially in the two risk groups.

3. Results

3.1. Literature Search and Characteristics of Included Studies

Of 6302 relevant search records, 107 independent articles were enrolled into systematic reviews (Fig. 1). During data extraction, 6195 articles were excluded, including 2680 duplicate articles, 2462 articles on animal or non-clinical experiments and 1053 articles that lacked available data. In 107 eligible studies, 151 datasets in total were utilized to produce their analyses (Additional file 1: Table S1).

The main characteristics of the eligible studies are summarized in Table 1 (Additional file 2–4: Table S2–S4). A total of 10486 patients, including 3662 patients with kidney cancer, 3971 patients with bladder cancer and 2853 patients with prostate cancer, were included. The median sample size was 87.5 (IQR: 50.25; range: 15–546). Of these recently published 107 articles (2009–2017), 68/107 studies came from Asia (60 of them were Chinese studies), followed by Europe (25/107), America (12/107) and Australia (1/107). According to the primary prognosis score, the datasets were divided into 3 groups and nearly half of the datasets were assigned to the favourable group (69/151). Of these eligible studies, approximately one-quarter of the datasets contained T

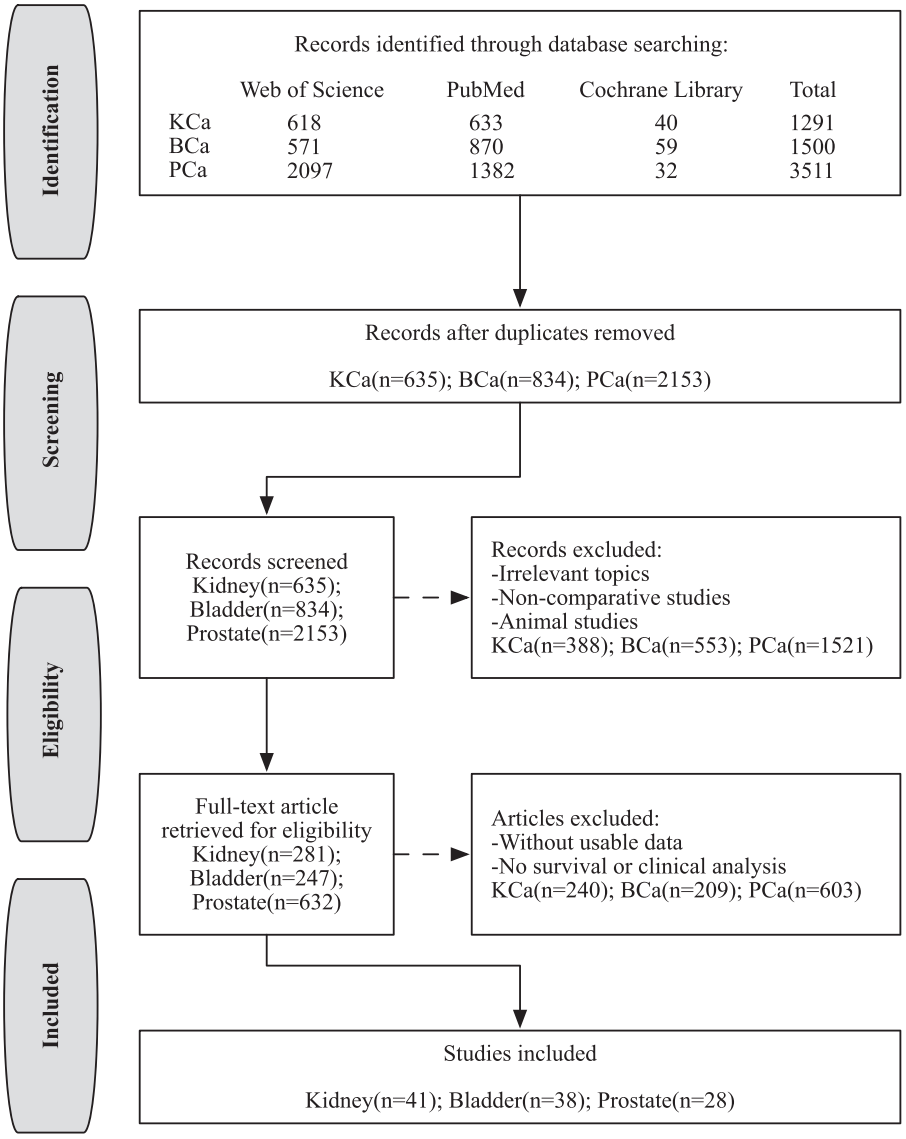


Fig. 1. A flow diagram depicting evidence acquisition and selection. Of the 3511 records, 107 were included in our systematic review.

Table 1
Descriptive statistics of eligible studies.

Characteristic	Subgroups	Frequency (%)
Cancer site	Kidney	41(38.31%)
	Bladder	38(35.51%)
	Prostate	28(26.17%)
	n	107(100.00%)
Year	2009	2(1.87%)
	2010	1(0.93%)
	2011	1(0.93%)
	2012	8(7.48%)
	2013	16(14.95%)
	2014	22(20.56%)
	2015	32(29.91%)
	2016	18(16.82%)
	2017	7(6.54%)
	n	107(100.00%)
Continent	America	12(11.21%)
	Australia	1(0.93%)
	Asia	68(63.55%)
	Europe	25(23.36%)
	Multiple	1(0.93%)
	n	107(100.00%)
Quantification method[a]	qPCR	4(3.74%)
	qRT-PCR	97(90.65%)
	ISH	4(3.74%)
	NA	2(1.87%)
	n	107(100.00%)
Sample type[b]	Frozen tissue	45(42.06%)
	Tissue	37(34.58%)
	FFPE	11(10.28%)
	Serum	6(5.61%)
	Urine	3(2.80%)
	NA	1(0.93%)
	Multiple	4(3.74%)
	n	107(100.00%)
Sample size	<50	25(23.36%)
	50–100	40(37.38%)
	>100	41(38.31%)
	NA	1(0.93%)
	n	107(100.00%)
Prognostic effect[c]	Favourable	69(45.70%)
	Moderate	36(23.84%)
	Unfavourable	46(30.46%)
	n	151(100.00%)
Clinical event[d]	T	38(27.54%)
	N	23(16.67%)
	M	20(14.49%)
	G	19(13.77%)
	PSA	14(10.14%)
	Gleason	12(8.70%)
	Stage	12(8.70%)
Survival event[e]	n	138(100.00%)
	OS	80(46.51%)
	DFS	18(10.47%)
	RFS	27(15.70%)
	CSS	24(13.95%)
	PFS	16(9.30%)
P value for survival analysis[f]	BCR-FS	7(4.07%)
	n	172(100.00%)
	P < .01	53(30.81%)
	P < .05	127(73.84%)
Variables analysis	P ≥ .05	45(26.16%)
	n	172(100.00%)
	Multivariate	65(67.71%)
Quality assessment[g]	Univariate	31(32.29%)
	n	96 (100.00%)
	3	1(0.93%)
	4	10(9.35%)
	5	5(4.67%)
	6	33(30.84%)
	7	21(19.63%)
	8	17(15.89%)
	9	20(18.69%)
	n	107(100.00%)

stage information; more than half of the datasets assessed OS, and most of the survival events (127/172) demonstrated statistical significance. Across the 65 studies that reported multivariable analyses, 42 adjusted for TNM stage, 37 adjusted for histologic grade and 25 adjusted for both (Additional file 5: Fig. S1). For prostate cancer, there were five studies adjusted for both PSA and Gleason score; another 16 studies just adjusted for PSA or Gleason score only. With respect to quality assessment, 100/107 studies were awarded a score of 6 or above.

Of particular interest, more than half of the studies were from China (60/107). We therefore tried to compare data from the Chinese (CHN) studies with non-Chinese (non-CHN) studies (Additional file 6–7: Table S5; Fig. S2). It is obvious that the CHN publications were more recent than were the non-CHN ones ($P < 0.01$). The statistical significance of the survival analyses in the CHN studies was more prominent than that in the non-CHN works ($P < 0.01$). There was also a significant difference in the prognostic effects ($P = 0.035$) and variable analyses ($P = 0.035$). However, there was no statistical significance in the cancer site, sample size or quality assessment between CHN and non-CHN studies.

3.2. Prognostic Effect of miRNAs

To highlight the prognostic effect of each miRNA in urologic cancer, we assigned the miRNAs to various effect subgroups (Additional file 8: Table S6). Some miRNAs showed a similar signature across multiple urologic cancers. For example, miR-21 had an unfavourable effect across three kinds of urologic cancer. Nevertheless, it was interesting that even the same miRNA in the same kind of cancer might have the different prognostic effects, such as miR-210, which had a favourable, moderate or unfavourable role in KCa. To further understand the effects of miRNAs in urologic cancer intuitively and integrally, we gathered the miRNAs that were reported twice or more and added their prognostic scores together (Table 2). Comparison among these miRNAs showed that nine miRNAs (miR-21, miR-214, miR-222, miR-34a, miR-129, miR-143, miR-200a, miR-26a and miR-27b) have high consistency on revealing the prognostic effect in urologic cancers. However, although there were many studies on miR-210 and miR-141, the consistency of the results is poor across the urologic cancers.

Inter- and intra-cancer group meta-analyses were performed for all miRNAs that were studied twice or more for the same prognostic event. For the intergroup analysis from KCa, BCa and PCa, 48 groups of datasets were included in the meta-analysis, of which 20 groups of meta-analyses were statistically significant and 28 were nonsignificant (Fig. 2; Additional file 9: Fig. S3). For the intragroup KCa or BCa or PCa, a total of 34 groups of datasets were included in the meta-analysis, only a half of these groups were statistically significant (Additional file 10: Fig. S4). miR-21, which was the most frequently studied miRNA and had high consistency among these studies, was significantly associated with an unfavourable prognostic effect among the 3 clinical events

Notes to Table 1:
These data are based on 151 datasets of 107 studies; n refers to the total number of observations for each characteristic; NA-not available.
[a] qPCR-quantitative polymerase chain reaction; RT-PCR-reverse transcription polymerase chain reaction; qRT-PCR-quantitative real-time polymerase chain reaction; ISH-in-situ hybridization.
[b] FFPE-formalin-fixed paraffin-embedded.
[c] Define according to the primary prognosis score (Favourable refer to score >0; Moderate refer to score = 0; Unfavourable refer to score <0).
[d] T-size or direct extent of the primary tumour; N-degree of spread to regional lymph node metastasis; M-presence of distant metastasis; G-histological grade; Gleason*-Gleason scores; PSA*-Prostate Specific Antigen (*prostate cancer only); Stage-TNM staging.
[e] OS-overall survival; RFS-relapse free survival; DFS-disease free survival; PFS-progression-free survival; CSS-cancer specific survival; BCR-FS-biochemical recurrence free survival.
[f] $P < 0.05$($P < 0.01$ included).
[g] The qualities of included studies were scored by Newcastle-Ottawa quality assessment scale (NOS).

and the 4 survival events in our meta-analyses, which demonstrated that the high expression of miR-21 was associated with a poor prognosis in three urologic cancers, whether in OS (HR:2.699, 1.76–4.14, $P < 0.001$), DFS (HR:1.865, 1.119–3.109, $P = 0.017$), PFS (HR:2, 1.318–3.034, $P = 0.001$) or CSS (HR:4.295, 1.43–12.901, $P = 0.009$) (Fig. 2; Table 3). To make the results more precise and persuasive, a meta-analysis of miR-21 in the same cancer was also performed and were also significantly associated with an unfavourable effect in both KCa and BCa. For miR-210, all inter- and intra-meta analyses were nonsignificant (Fig. 2; Additional file 10: Fig. S4). The prognostic signature of miR-210 indicated that it might play a moderate role in the prognosis of urologic cancer. Although only CSS was further meta-analysed in miR-141 and were nonsignificant, the general prognostic pattern suggested that miR-141 might play an unfavourable role in PCa and play a favourable role in BCa (Fig. 2).

For BCa subgroup, miR-100 (AT, OR:0.289, 0.112–0.746, P = .01), −200a (RFS, HR:0.524,0.311–0.885, $P = .016$) and − 214 (OS, HR:0.201, 0.126–0.319, $P < .001$) were significantly associated with a favourable effect, while miR-143 (PFS, HR: 3.779, 1.605–8.894, $P = .002$), −155 (PFS, HR: 8.102, 2.919–22.484, P < .001) and − 222 (OS, HR: 3.389, 1.103–10.415, P < .001) were significantly related to an unfavourable effect (Additional file 10: Fig. S4). For KCa subgroup, miR-126 (CSS, HR: 0.228, 0.13–0.399, P < .001) and -125b (CSS, HR: 2.473, 1.465–4.177, P < .001) were significantly associated with the prognostic effect (Additional file 10: Fig. S4). Besides, miR-205 (OS, HR: 0.403, 0.223–0.73, P < .001) played a favourable role in BCa and PCa. miR-23b (OS, HR: 0.227, 0.091–0.566, $P = .001$) were significantly associated with a favourable effect in BCa and KCa (Additional file 9: Fig. S3). After the meta-analyses, we adjusted the prognostic score of each miRNA according to its summary effects. Only if the meta-analysis displayed a nonsignificant summary effect was the corresponding score removed. The prognostic signatures of 12 miRNAs were refined (Additional file 11: Table S7) and the prognostic ability of miRNAs seem fluctuated.

Publication bias in this meta-analysis was evaluated with either Egger's test or Begg's test. No significant publication bias was found.

3.3. Integrated Analysis of Four Shared miRNAs in Urologic Cancer

A proving analysis was performed to verify the reliability of the above-mentioned miRNAs in urologic cancers. We generated a Venn diagram that calculates the intersections of the three cancers to summarize the shared miRNAs across urologic cancers in the original cohort (Fig. 3a). After getting an intersection, 4 miRNAs (miR-141, miR-203, miR-21 and miR-34a) were found in the three kinds of urologic cancers. The expression levels of these 4 miRNAs were confirmed in the TCGA cohort and nearly all of the shared miRNAs were aberrantly expressed in urologic cancer (Additional file 12: Fig. S5). To further validate the prognostic potential of the shared miRNAs in urologic cancer patients, we performed a Kaplan-Meier analysis of the TCGA cohort which demonstrated that only miR-34a-5p in KIRP, miR-141-3p in BLCA and miR-21-5p in PRAD were significantly associated with both OS and RFS and another few miRNAs were significantly correlated with OS or RFS. None of the shared miRNAs were simultaneously significantly associated with OS or RFS across the three cancers in the TCGA cohort. (Additional file 13–14: Table S8–9).

We further confirmed the correlations between the shared miRNAs and urologic cancer with an integrated meta-analysis of OS in the original and TCGA cohorts (Fig. 4). Generally, only miR-21 was significantly associated with OS in our integrated meta-analysis (HR:2.177,1.33–

Table 2
Prognostic score of miRNAs.

miRNA	Datasets	Fav/Mod/Unfav[a]	Sample size[b]	Events/P score(KCa)[c]	Event/P score(BCa)[d]	Event/P score(PCa)[e]	Event/P score(Total)
miR-21	13	0/5/8	77	15/−20	6/−9	5/−4	26/−33
miR-210	7	1/5/1	84	7/−2	2/0	0/0	9/−2
miR-141	6	4/1/1	67.5	3/+4	2/+4	4/−4	9/+4
miR-100	4	2/1/1	111	4/−6	10/+10	0/0	14/+4
miR-145	4	2/0/2	60.5	0/0	5/−3	5/+7	10/+4
miR-155	4	1/1/2	119.5	7/0	5/−7	0/0	12/−7
miR-200c	4	0/4/0	55	5/0	0/0	0/0	5/0
miR-203	4	2/0/2	67	9/+2	2/+4	1/−2	12/+4
miR-221	4	1/2/1	70	2/−2	0/0	2/+2	4/0
miR-27a	4	0/3/1	121.5	5/−7	2/0	0/0	7/−7
miR-205	3	2/1/0	49	0/0	2/0	1/+2	3/+2
miR-214	3	3/0/0	138	0/0	5/+10	0/0	5/+10
miR-222	3	0/1/2	97	0/0	4/−8	0/0	4/−8
miR-30a	3	1/2/0	62	2/0	3/+4	0/0	5/+4
miR-30c	3	2/1/0	44	1/+2	0/0	4/+4	5/+6
miR-34a	3	2/1/0	152	1/0	1/+2	1/+2	3/+4
miR-126	3	2/1/0	103	4/+4	0/0	0/0	4/+4
miR-452	2	1/0/1	43	0/0	1/−2	1/+2	2/0
miR-125b	2	0/0/2	138	14/−10	0/0	0/0	14/−10
miR-129	2	2/0/0	93.5	2/+4	0/0	5/+6	7/+10
miR-143	2	0/0/2	76.5	0/0	4/−8	0/0	4/−8
miR-182	2	0/1/1	103	0/0	4/−4	0/0	4/−4
miR-200a	2	2/0/0	132	0/0	2/+4	0/0	2/+4
miR-200b	2	1/0/1	66.5	0/0	3/−2	0/0	3/−2
miR-224	2	0/2/0	46	0/0	2/−2	1/0	3/−2
miR-23b	2	2/0/0	40.5	4/+4	1/+2	0/0	5/+6
miR-26a	2	2/0/0	72	1/+2	2/+4	0/0	3/+6
miR-27b	2	2/0/0	54	4/+4	0/0	1/+2	5/+6
miR-372	2	1/1/0	50	0/0	1/0	4/+1	5/+1
miR-429	2	0/2/0	64.5	2/0	0/0	0/0	2/0
miR-497	2	2/0/0	50.5	7/+6	0/0	0/0	7/+6

[a] Favourable versus moderate versus unfavourable.
[b] Sample size refer to the median size of the datasets.
[c] Prognostic events and total prognosis score in kidney cancer.
[d] Prognostic events and total prognosis score in bladder cancer.
[e] Prognostic events and total prognosis score in prostate cancer.

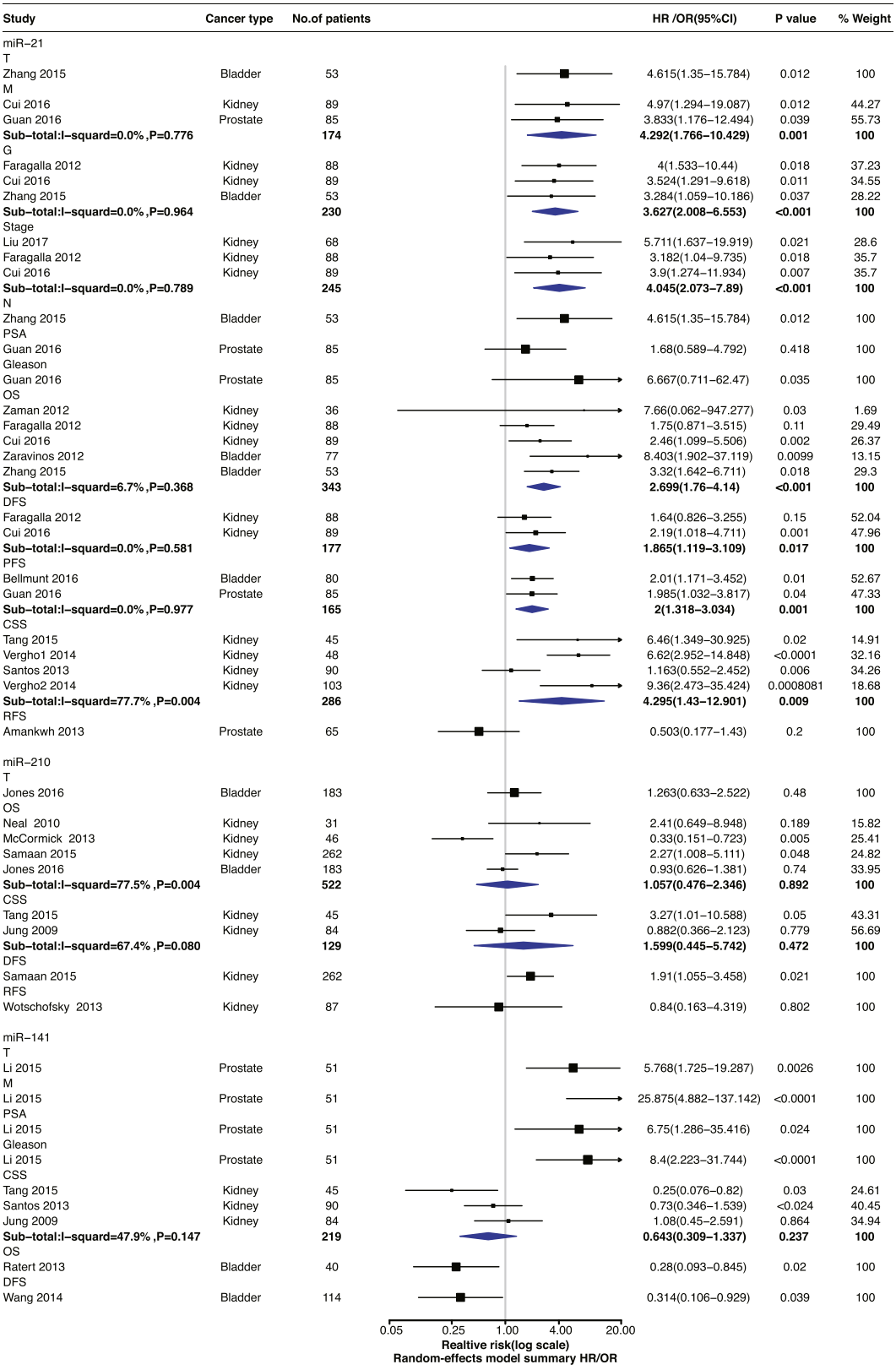


Fig. 2. General prognostic signatures and meta-analyses of miR-21, miR-210 and miR-141. All of the prognostic events of these miRNAs are illustrated in forest plots, with corresponding *P* value and 95% CI. We meta-analysed any events for which two or more studies were included. The estimated effect size of each event is presented as a black square that is proportional in size to the weight of the study. The pooled effect size is presented as a purple rhombus that is sized in the centre for summary effect size and whose width depicts the confidence interval. The confidence interval of effect size appears as a horizontal line (once the size exceeds this range, an arrow is placed), and the vertical line across these estimates represents HR = 1.

3.566, $P = 0.002$). The summary effects of miR-21 in the KCa subgroup were significant (HR: 1.998, 1.367–2.919, $P < 0.001$) and were consistent with the first-part of our meta-analysis. While the summary effects of miR-21in the BCa subgroup lacked significance in OS (HR: 2.458, 0.681–9.064, $P = 0.168$), which is different from what was observed in our primary meta-analysis. For the other three miRNAs, the overall and subgroup summary effects were statistically non-significant except for miR-203a in PCa subgroup. According to the integrated and reasonable subgroup analyses, we further refine the prediction ability of the 4 shared miRNAs in urologic cancer. Using single miRNAs as predictors of urologic cancer survival might still unreliable.

3.4. Creation of miRNA Panels and their Association with Urologic Cancer in TCGA

We identified numerous miRNAs among the three urologic cancers in the first part of our meta-analysis. Most of the eligible studies focused on one single miRNA or assessed multiple miRNAs separately, and only a few generated a prognostic analysis based on miRNA or gene integration [42, 43]. In order to provide a better stratification of expected survival, we summarized the diagnostic and prognostic miRNA panel in urologic cancer to explore the reported predictive miRNA panel (Additional file 15: Table S10). Two of the 20 panels were involved in both diagnostic and prognostic elements; the other 13/20 and 5/20 panels were only for diagnostic or prognostic, respectively. All the areas under the curve (AUCs), HRs and *P* values of each miRNA and panel were summarized. This summary showed that the panels displayed a better AUC and P value. We therefore tried to evaluate the prognostic power of the panels generated from the included miRNAs in the KCa group using the TCGA datasets. The KCa-35 and KCa-11 models indicated a better CI and HR, but not all of the miRNAs satisfied the eligible criteria (Table 4; Additional file 16: Fig. S6a-d). Six miRNAs were included in the final model (KCa-6: miR-27b, −942, −497, −144, −141 and -27a; CI,75.28; HR: 3.214, 1.971–5.240, $P < 0.01$). Survival risk curves are shown for each risk group (HR:3.30, 1.91–5.70, $P < 0.01$; Fig. 5a). Among these six differently expressed miRNAs (Fig. 5b), four had a favourable prognosis (miR-497, miR-141, miR-27b and miR-144), and two were associated with a high risk (miR-27a and miR-942). The OS analyses of each miRNA included in KCa-6 were also performed in the same TCGA-KIRC cohort individually (Additional file 16: Fig. S6e–j). Of particular interest is that only half of the included miRNAs were significantly associated with OS, with a worse stratification of patients' survival expectancy compared with KCa-6, indicating that a miRNA panel may contribute to a better risk stratification and prognostic prediction in patients with kidney cancer.

As excepted, relevant clinicopathologic characteristics predicted the prognosis of the patients in the TCGA-KIRC cohort (Table 4; Additional file 17: Fig. S7a-c). ROC curve analyses were performed to evaluate the predictive accuracy of histologic grade, pathologic T stage, pathologic stage (TNM stage) and KCa-6 (Fig. 5c). The AUC demonstrated that, from a prognostic point of view, the accuracy of KCa-6 (AUC,0.755) is comparable to that of pathologic stage (AUC,0.763) and is superior to those of histologic grading (AUC,0.721) and pathologic T stage (AUC,0.706). The combination of those four clinical covariates improved our ability to predict the prognosis of the same cohort (AUC,0.816). Further, the high-risk groups (G3/G4, T3/T4 and stage III/IV) had a higher prognostic index, which indicated a poorer prognosis (Additional file 17: Fig. S7d-f). However, only pathologic stage and KCa-6 were significantly associated with OS in the final multivariate analysis model (Table 4). Kaplan–Meier curves showed that patients separated by both KCa-6 and staging have significantly different prognoses ($P < 0.0001$; Fig. 5d). It is therefore reasonable that the novel appropriate combination of miRNAs and the current predictive factor would enable the more efficient construction of a prognostic model Table 5.

4. Discussion

The current knowledge of miRNA function in urologic tumours has caught our attention. The particular promise of miRNAs in the diagnosis, prognosis and treatment of urologic tumours has been reflected in an increasing number of publications [13, 44]. Benefitting from an advanced research effort, more aberrant miRNAs have been distinctly identified and associated with relevant clinicopathologic characteristics, including prognosis. For example, miR-143/145 cluster serves as a robust prediction marker of oncologic outcome for BCa patients [42]. miR-221 constitutes a novel prognostic biomarker in high-risk prostate cancer [45]. As more studies with statistically significant results have been reported, many reviewers have attempted to summarize the potential association between the miRNA expression levels and urologic cancer prognosis [27, 29, 30]. However, it is difficult to carry out a sensible and meaningful meta-analysis without having access to individual patient data due to the well-recognized problems with systematic reviews of prognostic studies, such as poor methodologic quality, potential publication bias, a wide heterogeneity in many aspects, and inadequate reporting of quantitative information [1, 46]. The previous

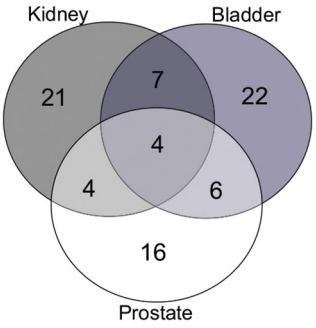
Table 3
The results of meta-analysis for miR-21 among three urologic cancers.

	No.of miRNA Dataset	No.of patients	HR/OR (95%CI)[h]	P[i]value	Heterogeneity[j]	
					I^2(%)	P value
Clinical						
M[a]	2	174	4.292(1.766-10.429)	0.001	0.0	0.776
G[b]	3	230	3.627(2.008–6.553)	<0.001	0.0	0.964
Stage[c]	3	245	4.045(2.073–7.89)	<0.001	0.0	0.789
Survival						
OS[d]	5	343	2.699(1.76–4.14)	<0.001	6.7	0.368
DFS[e]	2	177	1.865(1.119–3.109)	0.017	0.0	0.581
PFS[f]	2	165	2(1.318–3.034)	0.001	0.0	0.977
CSS[g]	4	186	4.295(1.43–12.901)	0.009	77.7	0.005

[a] Presence of distant metastasis.
[b] Histological grade(G3-G4).
[c] TNM Staging (Stage III-IV).
[d] Overall survival.
[e] Disease free survival.
[f] Progression-free survival.
[g] Cancer-specific survival.
[h] HR-hazard ratio; OR-odds ratios; CI-confidence interval.
[i] P value for summary effect of prognostic events.
[j] I^2 and *P* value measure of between-study heterogeneity.

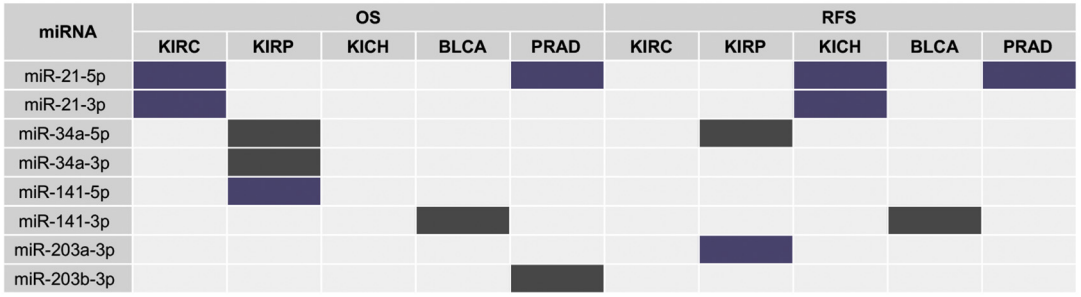
prognostic meta-analysis also demonstrated that the estimates of heterogeneity metrics had wide 95% confidence intervals [47]. Methodologic guidelines, such as the BRISQ [48](Biospecimen Reporting for Improved Study Quality), REMARK [49] (Reporting Recommendations for Tumour Marker Prognostic Studies) and TRIPOD [50] (Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis) metrics, have been repeatedly proposed to improve the reporting of these types of studies [51], but it is still unknown if any will be promoted widely and used properly [52]. We therefore attempted to integrate a meta-analysis and a bioinformatics analysis to better explore the role of prognostic miRNAs in urologic cancer.

a.



Group	Total	miRNAs
Bladder Kidney Prostate	4	miR-21 miR-34a miR-203 miR-141
Bladder Kidney	7	miR-155 miR-210 miR-100 miR-30a miR-23b miR-27a miR-26a
Kidney Prostate	4	miR-27b miR-221 miR-30c miR-129
Bladder Prostate	6	miR-452 miR-145 miR-205 miR-222 miR-372 miR-224
Kidney	21	miR-497 miR-628 miR-429 miR-630 miR-506 miR-200c miR-1 et.al
Bladder	22	miR-486 miR-200b miR-576 miR-223 miR-3713 miR-143 miR-9 et.al
Prostate	16	miR-503 miR-132 miR-301a miR-150 miR-135a-1 miR-19a miR-195 et.al

b.



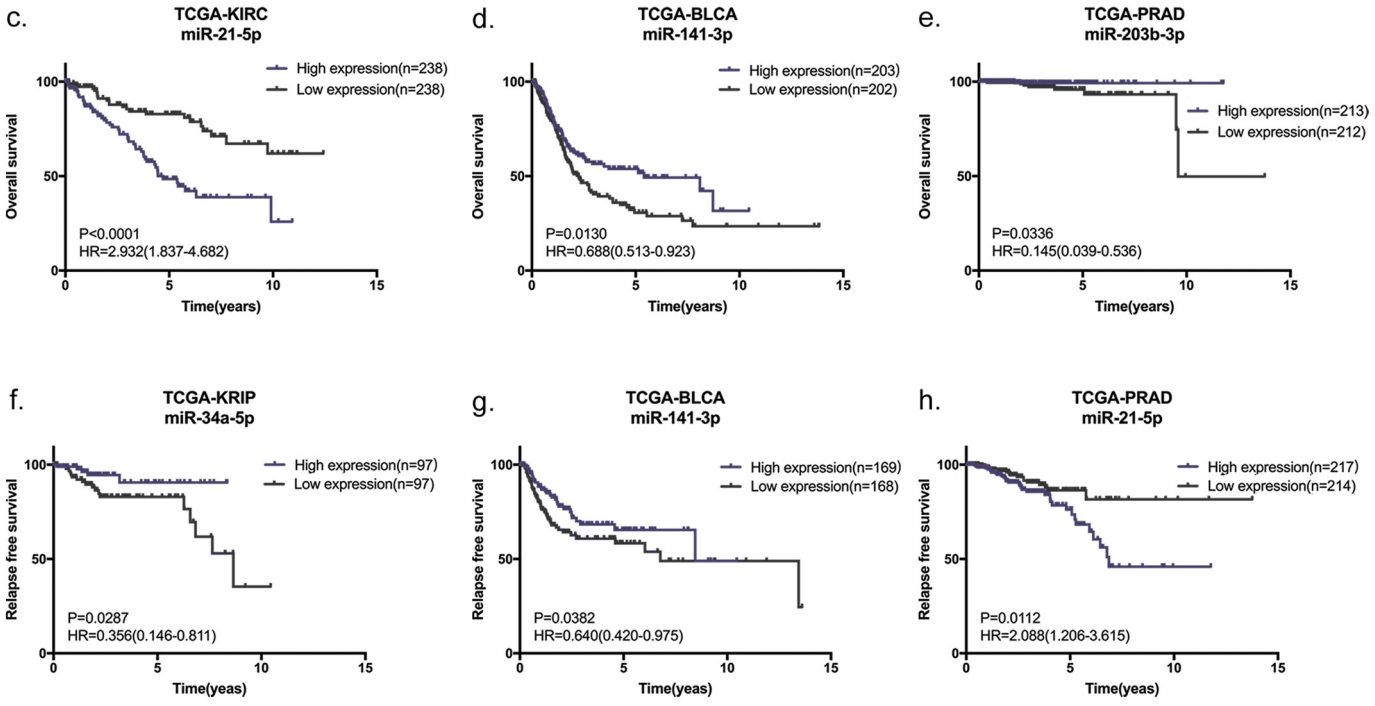


Fig. 3. The prognostic signatures of miR-21, miR-34a, miR-141 and miR-203 in the TCGA cohort. a. A Venn diagram of consensus miRNAs across three urologic cancers. b. A data microarray of the prognostic effects of 4 miRNAs on OS and RFS. Red = unfavourable prognostic effect ($P < .05$, HR > 1); Blue = favourable prognostic effect ($P < .05$, HR < 1); White grey = moderate prognostic effect ($P \geq .05$). c-e. Representative Kaplan-Meier curves of the 4 miRNAs on OS with a significant prognostic effect. f-h. A representative Kaplan-Meier curve of the 4 miRNAs on RFS with a significant prognostic effect.

Similar to previous studies, the problems with systematic reviews of prognostic studies were prominent in our meta-analysis. Most of eligible studies in our collection were Chinese (60/107 in all, 60/68 in the Asia subgroup). On the one hand, the year-by-year number of Chinese articles in the field of urology has increased substantially over the past decade, although the quality of these papers still needs to be improved [53]. On the other hand, in a previous analysis on genome epidemiology, the papers from China showed evidence of significantly more prominent genetic effects than non-Chinese studies with smaller sample sizes [54]. Our study also found that the CHN studies showed more significant effects than non-CHN studies did. It is well known that, both theoretically and empirically, smaller studies are associated with larger heterogeneity [55] and inflated effect size estimates [56, 57]. Less than one-third of the eligible datasets (49/152) in our primary meta-analysis had a sample size of >100. However, of particular interest is that the sample sizes of the Chinese studies were nearly the same as those of the non-Chinese studies. That is, not only was there a limited sample size, but worse methodological quality, publication bias and selection bias, called the 'small-study effect' all contributed to inflated effect size estimates [58].

Unlike the previous meta-analysis, which focused on only a single clinical event or survival analysis, we have tried to gather all kinds of survival analyses and clinical events involved in prognosis. It is well known that clinicopathologic characteristics, such as TNM stage, histologic grade, PSA and Gleason score, are closely related to cancer prognosis. A meta-analysis of these clinical events in miRNA can therefore

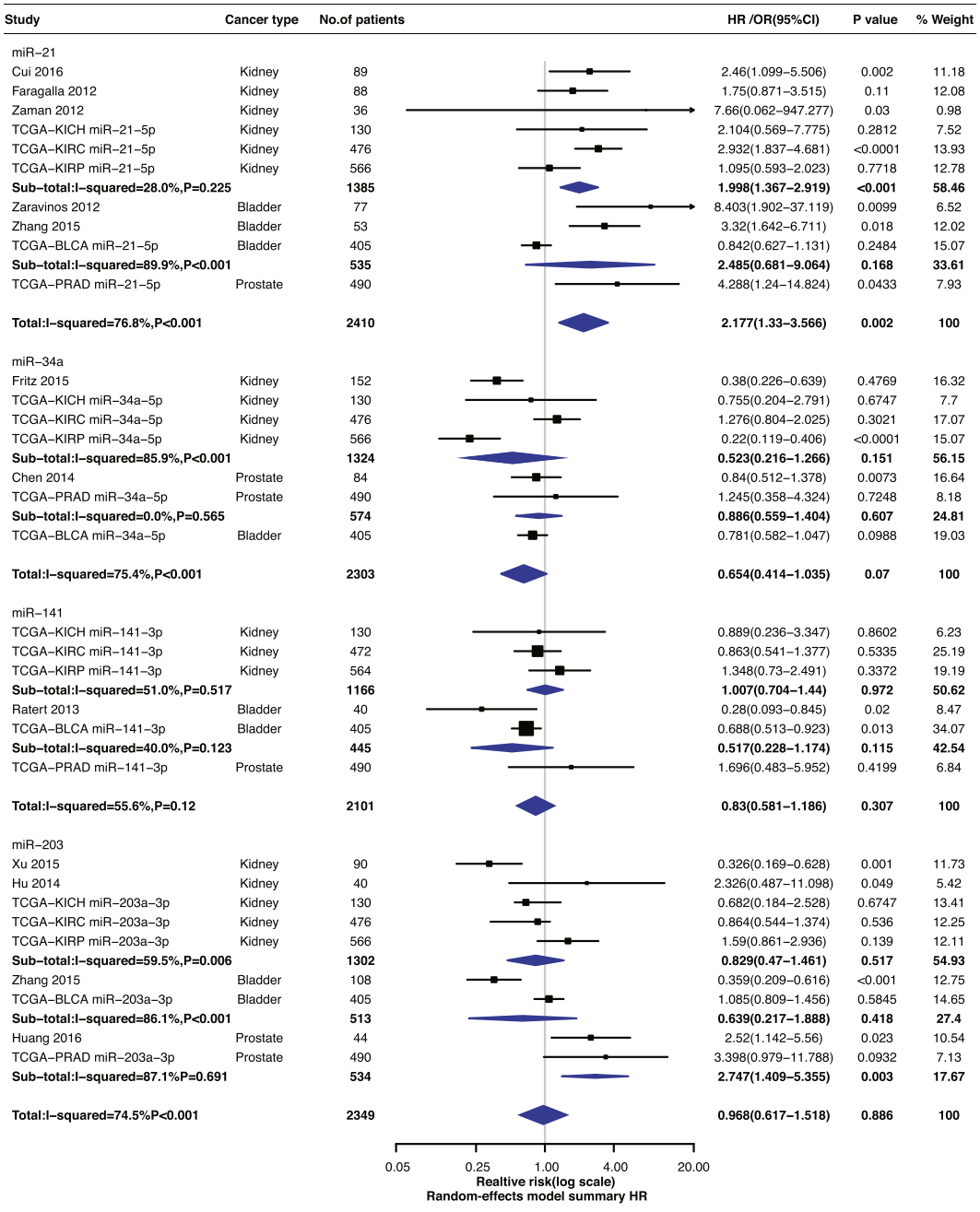


Fig. 4. Integrated meta-analyses of miR-21, miR-34a, miR-141 and miR-203 in OS. All of the OS of these miRNAs are illustrated in forest plots, with corresponding *P* values and 95% CIs. The estimated effect size of each event is presented as a black square proportional in size to the weight of the study. The pooled effect size is presented as a purple rhombus in size with centre for summary effect size and with width for its confidence interval. The confidence interval for the effect size appears as a horizontal line (once its size exceeds the range, an arrow is placed), while the vertical line across these estimates represents HR = 1.

Table 4
The result of Cox model for three miRNA panel in TCGA-KIRC cohort.

miRNA panel	CI[a]	Risk group P value	Risk group HR(95%CI)	β_i[b] > 0/β_i < 0	P(β_i)[c] < 0.05 N	DEG[d] N
KCa-35	81.05	7.44E-12	7.215(4.417–11.790)	17/18	9	14
KCa-11	76.73	2.32E-07	3.873(2.374–6.320)	5/6	11	8
KCa-6*	75.28	0.000004	3.214(1.971–5.240)	2/4	6	6
6*-1	mir-27b	0.000087	0.361 (0.223–0.586)	–	–	–
6*-2	mir-942	0.0275	1.707 (1.050–2.774)	–	–	–
6*-3	mir-497	0.0047	0.496 (0.306–0.805)	–	–	–
6*-4	mir-144	0.0729	0.637 (0.392–1.033)	–	–	–
6*-5	mir-27a	0.3077	1.285 (0.793–2.083)	–	–	–
6*-6	mir-141	0.1192	0.684 (0.421–1.111)	–	–	–

[a] Concordance Index.
[b] β_i coefficient in Cox Proportional Hazard regression.
[c] Wald test P value (Cox Fitting).
[d] Differential expressed genes.

reveal comprehensive associations between miRNA and prognosis. We first scored all of the eligible miRNAs according to their prognostic events to attempt to understand the general miRNA signature among urologic cancers. Those miRNAs that were studied two or more times were highlighted and involved in our meta-analysis. A series of statistically significant prognostic miRNAs have been summarized. For instance, miR-143, −155 in BCa, miR-125b, −126 in KCa, miR-21 in three urologic cancers and so on. Among these, therapeutic manipulation based on miR-143 [59] and miR-155 [60, 61] in preclinical models have already been reported. Even more, the clinical trial of antimiR-155 (MRG-106; miRagen Therapeutics) in patients with cutaneous T cell lymphoma and mycosis fungoides subtype were initiated. The clinical value of these miRNAs may not only be limited to the prediction of prognosis, but also have the potential to be transformed into therapeutics.

Although combining various prognostic factors and survival analyses could better evaluate the prognosis of urologic cancers, our analyses were still limited by individual data acquisition and the insufficient number of studies on each parameter in each miRNA. We therefore tried to integrate the TCGA cohort into a further meta-analysis, and 4 shared miRNAs were included. Only miR-21 was significantly associated with prognosis. Interestingly, compared with the first-part of the meta-analysis, different results were found in the integrated meta-analysis, such as the prognostic effect of miR-21 in BCa. miR-21, which is one of the most studied miRNAs, is expressed aberrantly and functions as a vital regulator in a broad range of cancers, thereby meeting the rigorous criteria of an ideal biomarker for use in the diagnosis and management of cancer [62]. Quite a few recent meta-analyses have summarized the prognostic role of miR-21 in various cancers, and they clearly indicated that miR-21 can predict an unfavourable prognosis [63, 64]. Similar

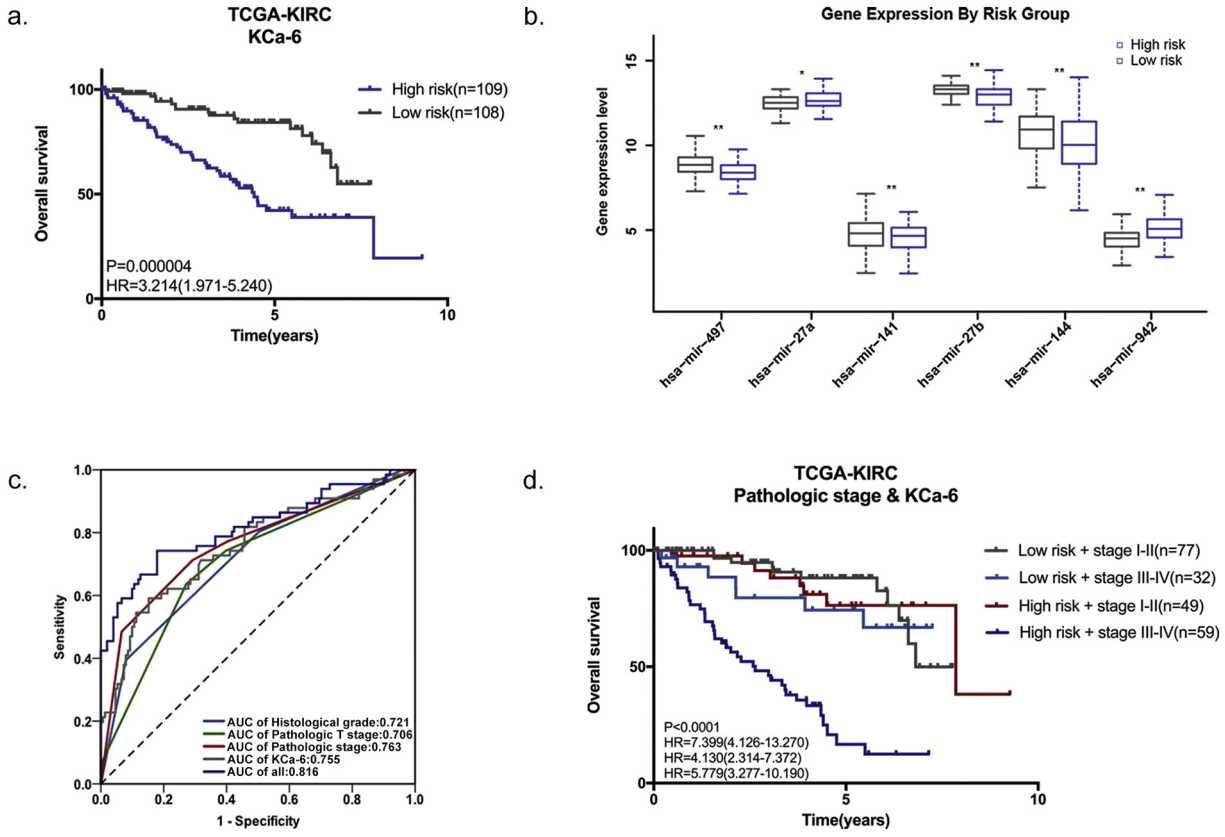


Fig. 5. The miRNA panel KCa-6 for predicting KIRC survival. a. The overall survival curve of KIRC patients with low or high risk, according to KCa-6. b. The expression level of each miRNA included in KCa-6. **: $P < .01$. c. The ROC for pathologic T stage, histologic grade, pathologic stage, KCa-6 and their combination. d. Survival curves of KIRC patients that combine KCa-6 risk and staging.

Table 5
Univariate and multivariate analyses of clinicopathological characteristics and KCa-6 miRNA panel with overall survival.

Variables	AUC[a]	Univariate analysis[c]		Multivariate analysis[d]	
		HR[b] (95%CI)	P value	HR(95%CI)	P value
Histological grade	0.721	2.692(1.644–4.408)	0.000844	–	–
Pathologic T stage	0.706	3.730(2.236–6.221)	1.1353E-07	–	–
Pathologic stage	0.763	4.502 (2.719–7.454)	9.0589E-10	4.065 (2.331–7.092)	7.4469E-07
KCa-6	0.755	3.214 (1.971–5.240)	0.000004	2.710(1.553–4.739)	0.000449

[a] Area under the curve.
[b] Hazard ratio.
[c] Univariate analysis were performed by Kaplan-Meier estimator (log-rank test).
[d] Multivariate analysis used backward method and removal of 4 clinical covariates found to be associated with survival in univariate models ($P < 0.05$) and final model include only 2 covariates that were significantly associated with survival (Wald test, $P < 0.05$).

results were also confirmed in the first part of our meta-analysis. Our meta-analysis of miR-21 demonstrated that a higher expression level of miR-21 contributes to a poorer urologic cancer prognosis. However, a statistically significant association between miR-21 and OS was confirmed in only the TCGA-KIRC and TCGA-PRAD cohorts. Therefore, integrating a parallel TCGA analysis would allow the unbiased and objective assessment of the causal relationships between miRNA expression and OS.

Of our eligible studies, most focused on a single miRNA or multiple miRNAs separately. Only 4/107 generated a prognostic analysis based on miRNA or gene integration. miRNA always serves as a valuable source of biomarkers because of its highly dynamic expression pattern in various cancers, but we are still unable to predict cancer prognosis with a single miRNA due to its limited sensitivity and specificity. Thousands of aberrant miRNAs are involved in each type of cancer. Different types of cancers always share a similar miRNA signature [65]. Meanwhile, interpatient and intratumoural heterogeneity are general cancer characteristics, and miRNAs are no exception [66, 67]. It is therefore more reasonable and more precise to apply the signature of multiple miRNAs to predict tumour prognosis. According to our review of the miRNA panel, these panel had better a predictive ability of patients' expected survival. Similar effects were also confirmed in the miRNA panels generated from the included miRNAs. The predictive accuracy of the miRNA panel KCa-6 was nearly the same as that of pathologic stage. To ascribe some utility to the miRNA panel, the panel was adjusted for classical prognostic factors (e.g., histologic grade, pathologic T stage, pathologic stage). Surprisingly, only pathologic stage and KCa-6 were included in the final multivariate analysis, and the patients separated by these factors had significantly different prognoses. Adjusting and combing the gene panel for known prognostic factors will definitely contribute to clinical practice.

Our integrated analysis has several limitations. First, it is possible that relevant studies may have been missed, as our systematic review was based on only 3 databases (PubMed, Cochrane Library and Web of Science). Theoretically, we could have searched for possible missing articles in grey literature, relevant books, databases such as Google Scholar and other potential resources. In that way, our search strategy would be more sufficient, and the risk of publication bias would be reduced. Second, the annotation and nomenclature of miRNA were not unified across the included studies. Some named the miRNA according to their relative abundances, while others named the miRNA according to their derivation from the 3′ or 5′ arm [68]. Although we normalized the ID of the miRNA with miRBase [35] before the analysis, it is possible that some of the miRNAs might not match if the author used an unofficial annotation system. Third, this is a literature-based meta-analysis without individual patient data. Although we had applied a subgroup analysis to mitigate potential heterogeneity, it still seemed to persist across the different part of meta-analysis. This may have been the result of the combination of multiple studies that are known to be heterogeneous in terms of cancer site, patient origin, cut-off types and a small sample size due to the limited number of included studies for each parameter in each miRNA. Also, this is a literature-based meta-analysis without individual patient data. Use of individual patient data may further reduce these uncertainties of the estimates. Thus, regarding further proving analysis, we tried to use data from publicly available data sets, which might mitigate the related limitations. Fourth, 47/152 datasets did not provide the most accurate direct estimate of HR, in which case, we had to extract the data ourselves from the Kaplan–Meier curves. Even though this is accepted practice and has been widely used, the result might still be not sufficiently accurate due to the inevitable error introduced by the extracting process.

In conclusion, we have gathered almost all of the prognostic data regarding the association between miRNA and urologic cancer. The robustly unfavourable prognostic effect of miR-21 has been highlighted across three urologic cancers in the primary analysis. However, the effect of miR-21 in BCa fluctuated in the further confirmation demonstrating single miRNA still lacks the stability. miRNA panel contribute to a better risk stratification and prognostic prediction which could compensate for the unreliability of individual miRNAs in estimating prognosis. However, larger studies with a standardized methodology that assess both single and multiple miRNAs will offer better insight into the prognostic value of miRNAs in urologic cancer.

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Declaration of Interests

The authors declare no conflict of interest.

Authors' Contributions

Z.C: conception and design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content; Y.Z: conception and design, drafting of the manuscript; S.G: acquisition of data, drafting of the manuscript; X.Z: analysis and interpretation of data, drafting of the manuscript; Z.C, J.C and A.H statistical analysis; J.Z and Y.H: acquisition of data; J.C and Y.G: critical revision of the manuscript for important intellectual content; X.L and L.Z: critical revision of the manuscript for important intellectual content, administrative support,obtaining funding, supervision.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

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Appendix A. Supplementary data

All data generated or analysed during this study are included in this published article and its Additional file information files. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.07.014.

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